

# CONTENTS

Chapter No.	Title	Page No.
	<b>LIST OF TABLES</b>	
	<b>LIST OF FIGURES</b>	
	<b>ABSTRACT</b>	
<b>1</b>	<b>INTRODUCTION</b>	<b>1 – 7</b>
<b>2</b>	<b>REVIEW OF LITERATURE</b>	<b>8 – 44</b>
	2.1 Scenario on cancer	8
	2.2 Lung cancer - The study of concern	9
	2.3 Epidemiology of lung cancer	12
	2.4 Treatment strategies for lung cancer	14
	2.5. Modern approaches in treating lung cancer	15
	2.6 Apoptosis	17
	2.7 Plants as therapeutic agents for cancer treatment	19
	2.8 <i>Plectranthus amboinicus</i> - The candidate plant	20
	2.9 Pharmacological properties of <i>P. amboinicus</i>	21
	2.10 Metabolite profiling of bioactive phytocompounds	30
	2.11 Syringic acid – The compound of study	32
	2.12 Therapeutic applications of syringic acid	34
	2.13 <i>In silico</i> studies on lung cancer targets	38
	2.14 A549 lung cancer cell lines – An overview	41
	2.15 Mice model in cancer research	43
<b>3</b>	<b>MATERIALS AND METHODS</b>	<b>45 – 66</b>
	3.1 Phytochemical screening of <i>P. amboinicus</i> leaf extracts	45
	3.1.1 Collection and preparation of leaf extracts	47
	3.1.2 Preliminary phytochemical screening in the leaf extracts	48
	3.2 Determination of free radical scavenging activity in the methanolic extract of <i>P. amboinicus</i> leaves	48
	3.2.1 Determination of DPPH radical scavenging activity	49
	3.2.2 Determination of ABTS radical scavenging activity	49
	3.2.3 Determination of hydrogen peroxide scavenging activity	49
	3.2.4 Determination of inhibition of super oxide radical generation	49

Chapter No.	Title	Page No.
	3.2.5 Determination of inhibition of nitric oxide radical generation	49
3.3	In silico docking of phytochemicals from <i>P. amboinicus</i> with apoptotic targets	49
	3.3.1 Identification of phytochemicals in <i>P. amboinicus</i> by GC-MS analysis	49
	3.3.2 Ligand generation	50
	3.3.3 Drug likeness and pharmacokinetic properties	50
	3.3.4 Receptors preparation and molecular docking	50
	3.3.5 Density Functional Theory analysis	53
	3.3.6 Bioactivity score prediction	53
3.4	Identification of bioactive principles in <i>P. amboinicus</i>	54
	3.4.1 FT- IR analysis in the methanolic extract of <i>P. amboinicus</i>	54
	3.4.2 NMR analysis in the methanolic extract of <i>P. amboinicus</i>	54
	3.4.3 Extraction of syringic acid fraction by bioassay guided fractionation	54
	3.4.4 UV- Vis spectral analysis in the isolated fraction	55
	3.4.5 HPLC analysis in the isolated fraction	55
	3.4.6 HPTLC analysis in the isolated fraction	56
3.5	Cytotoxic activity of syringic acid fraction against A549 human lung cancer cells - An in vitro approach	56
	3.5.1 Cell lines and culture conditions	56
	3.5.2 Treatment and experimentation	57
	3.5.3 Evaluation of cytotoxicity by MTT dye reduction assay	57
	3.5.4 Detection of nuclear changes associated with apoptosis	58
	3.5.5 Determination of apoptosis by Annexin V/FITC flow cytometry	58
	3.5.6 Analysis of cell cycle arrest by flow cytometry	59
	3.5.7 Apoptotic protein expression by Western blotting	59
	3.5.7 Apoptotic gene expression by Reverse transcription - polymerase chain reaction (RT- PCR)	60

Chapter No.	Title	Page No.
	3.6 Anticancer activity of syringic acid fraction in benzo(a)pyrene induced experimental mice – An in vivo approach	60
	3.6.1 Selection and maintenance of the animals	60
	3.6.2. Determination of acute toxicity study	61
	3.6.3 Induction of tumor in experimental mice	61
	3.6.4 Experimental design	62
	3.6.5 Evaluation of syringic acid fraction on tumour growth response in experimental mice	63
	3.6.5.1 Mean survival time	63
	3.6.5.2 Increased Life Span	63
	3.6.5.3 Body weight analysis	63
	3.6.5.4 Relative lung weight	63
	3.6.6. Analysis of haematological and biochemical parameters	64
	3.6.7 Histopathological studies of the lung tissues of experimental animals	65
	3.6.8. Statistical analyses	66
<b>4</b>	<b>RESULTS AND DISCUSSION</b>	<b>67 - 144</b>
	4.1 Phytochemical screening of P. amboinicus leaf extracts	67
	4.2 Free radical scavenging activity in the methanolic extract of P. amboinicus leaves	67
	4.2.1 Determination of DPPH radical scavenging activity	67
	4.2.2 Determination of ABTS radical scavenging activity	67
	4.2.3 Determination of hydrogen peroxide scavenging activity	67
	4.2.4 Determination of inhibition of super oxide radical generation	67
	4.2.5 Determination of inhibition of nitric oxide radical generation	67
	4.3 In silico docking of phytocompounds from P.amboinicus with apoptotic targets	67
	4.3.1 GC-MS analysis in of P. amboinicus leaf extract	67
	4.3.2 Drug likeness and pharmacokinetic properties	67
	4.3.3 Receptors preparation and molecular docking	67
	4.3.4 Density Functional Theory analysis	67
	4.3.5 Bioactivity score prediction	67

Chapter No.	Title	Page No.
4.4	Identification of bioactive principles in <i>P. amboinicus</i> leaf extract	67
4.4.1	FT-IR analysis in the methanolic extract of <i>P. amboinicus</i>	67
4.4.2	NMR analysis in the methanolic extract of <i>P. amboinicus</i>	67
4.4.3	Syringic acid fraction extraction by bioassay guided fractionation	68
4.4.4	UV-Vis spectral analysis of the syringic acid fraction	68
4.4.5	HPLC analysis of the syringic acid fraction	68
4.4.5	HPTLC analysis in the syringic acid fraction	68
4.5	Cytotoxic activity of syringic acid fraction against A549 human lung cancer cells – An in vitro approach	68
4.5.1	Cytotoxic activity of A549 cells by MTT dye reduction assay	68
4.5.2	Nuclear change analyses by AO/EtBr, DAPI and PI staining	68
4.5.3	Detection of apoptotic events in A549 cells	68
4.5.4	Determination of cell cycle analysis by Annexin V/FITC staining	68
4.5.5	Regulation of protein and gene expression by Western Blotting and RT - PCR	68
4.5.6	Mechanism of SAF in the activation of apoptosis	68
4.6	Anticancer activity of syringic acid fraction in benzo(a)pyrene induced experimental mice – An in vivo approach	68
4.6.1	Acute toxicity study	68
4.6.2	Effect of syringic acid fraction on tumor growth factors	68
4.6.2.1	Mean survival time	68
4.6.2.2	Increase life span	68
4.6.2.3	Analysis of body weight	68
4.6.2.4	Analysis of relative lung weight	68
4.6.3	Effect of syringic acid fraction on haematological parameters	68

---

<b>Chapter No.</b>	<b>Title</b>	<b>Page No.</b>
	4.6.4 Effect of syringic acid fraction on liver marker enzymes	68
	4.6.5 Effect of syringic acid fraction on renal markers	68
	4.6.6 Effect of syringic acid fraction on tumor marker enzymes	68
	4.6.7 Histopathological studies of the lung tissues	68
<b>5</b>	<b>SUMMARY AND CONCLUSION</b>	<b>145 - 150</b>
	<b>REFERENCES</b>	<b>151 - 197</b>
	<b>APPENDICES</b>	<b>198 - 221</b>
	<b>PLAGIARISM REPORT</b>	

---